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Title: **Seroepidemiology of Human Cytomegalovirus and Human Herpesvirus 6 in a Cohort of Healthy Blood Donors from Abbottabad, Pakistan**

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Seroepidemiology of Human Cytomegalovirus and Human Herpesvirus 6 in a Cohort of Healthy Blood Donors from Abbottabad, Pakistan

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ABSTRACT

Background. Human Cytomegalovirus (CMV) and Human Herpesvirus 6 (HHV-6) represent significant public health concerns due to their widespread prevalence and potential clinical sequelae. This study aimed to elucidate the sero-epidemiological profile of CMV and HHV-6 among a cohort of ostensibly healthy blood donors in Abbottabad, Pakistan.

Methods. A cross-sectional study was conducted from December 2021 to June 2022 at the Regional Blood Centre in Abbottabad. Initially, 1850 healthy male blood donors were recruited according to WHO criteria, with 1750 meeting eligibility after screening for high-risk behaviors and clinical symptoms. Plasma samples were assayed for anti-CMV IgG, CMV IgM, and HHV-6 IgM using ELISA kits (sensitivity: 99%, specificity: 95%), with optical density measured at 450/620 nm. Donors were stratified into four age groups (<18, 21–30, 31–40, and 41–50 years) and statistical analyses were performed using descriptive statistics and Pearson's Chi-square test ($p<0.05$) in SPSS (version 25). Of the 1850 initially recruited donors, 1750 met the inclusion criteria (mean age: 28.2 years; range: 19–50 years).

Results. Initial screening revealed low prevalence rates for HBsAg (0.69%), anti-HCV (2.4%), and syphilis (1.14%), with all donors testing negative for malarial parasites and HIV. Blood group distribution was predominantly O (36%) and B (36%), with 96% of donors being Rh-positive. Overall, serological assessment demonstrated a CMV IgG seroprevalence of 90.2%, CMV IgM positivity in 5.7%, and HHV-6 IgM positivity in 8% of donors. Age-stratified analysis indicated: donors aged <18 years exhibited 80% CMV IgG positivity (with no CMV IgM or HHV-6 IgM), those aged 21–30 years 89% CMV IgG, 5.45% CMV IgM, and 9.1% HHV-6 IgM positivity; donors aged 31–40 years showed 94.2% CMV IgG, 7.69% CMV IgM, and 7.6% HHV-6 IgM positivity; while donors aged

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41–50 years demonstrated universal CMV IgG positivity without detectable CMV IgM or HHV-6 IgM.

Conclusion. The elevated CMV IgG seroprevalence among Abbottabad blood donors indicates widespread viral exposure, while the lower rates of CMV IgM and HHV-6 IgM suggest infrequent recent or reactivated infections. These findings underscore the need for continued sero-epidemiological surveillance to inform and optimize regional blood safety protocols.

Keywords: blood donors, blood transfusion, Cytomegalovirus (CMV), Human Herpesvirus-6 (HHV-6), Pakistan, Transfusion-Transmitted Infections (TTIs)

Highlights

- Cytomegalovirus (CMV) and Human Herpes Simplex-6 (HHV-6) are common in the blood of healthy donors in Abbottabad, with a significant number of donors testing positive for CMV IgG antibodies.
- The presence of CMV and HHV-6 IgM antibodies was attested in a remarkable percentage of donors, including the highest prevalence in the 21-30 age group, eliciting apprehensions about their putative transmission through blood transfusion.
- The findings emphasize the need to incorporate testing for these infections as a part of routine pre-transfusion screening.

1. INTRODUCTION

Every year thousands of lives are saved by blood transfusions, worldwide. Yet, unsafe transfusion raises the risk of Transfusion-Transmitted Infections (TTIs) and has multiple life-threatening consequences [1]. TTIs compromise blood safety and result in significant public health issues. An infection might still spread from a healthy individual when it is asymptomatic. To save the life of a critically ill recipient through blood transfusion, transfusion-transmitted pathogens must be screened before the transfusion process [2]. Cytomegalovirus (CMV) and Human Herpes Simplex Virus-6 (HHV-6) are transfusion-transmitted pathogens that establish latency and pose significant risks to immunocompromised hosts, transplant recipients, and pregnant individuals [3]. In immunocompromised individuals, HHV-6 can cause a spectrum of clinical manifestations including unexplained fever, rash, hepatitis, pneumonitis, encephalitis, and bone marrow suppression [4], while CMV infections typically present with systemic symptoms such as malaise, fever, and fatigue, with particular severity in pregnant women, potentially leading to miscarriage and congenital abnormalities [5].

HHV-6 transmission involves virus dissemination via saliva and respiratory excretions and it can be transmitted also through blood transfusion [4]. Recent studies indicate that organ transplantation may serve as an additional transmission route, supplementing sexual transmission. Furthermore, HHV-6—comprising strains A and B—can integrate into both somatic and germ cell chromosomes, resulting in inherited chromosomally integrated HHV-6 in approximately 1% of the global population (~70 million individuals) [3, 6].

CMV transmission can occur both vertically and horizontally after contact with contaminated body fluids, including

blood. A significant route of infection for high-risk groups is through the transfusion of blood products from latently infected donors [7]. CMV is a major transfusion-transmitted pathogen that can result in severe complications among immunocompromised recipients [8]. According to a survey, the global estimation of CMV in the general population is 83%, while in blood donors it is 84%, and among women of childbearing age it is 86% [9]. The frequency of HHV-6 in blood donors varies significantly across regions, ranging from a lower prevalence of 6.1% in Ouagadougou [10], to a higher rate of 71.7% in Qatar [4], and 78.8% in Greece [11].

Serological assessments are at hand to establish the presence of CMV and HHV-6 IgG. Similarly, IgM indicates recent infection in patients either with CMV or HHV-6. Many assays can detect antibodies but ELISA is mainly used to detect CMV and HHV-6 [4, 12]. This advanced technique enables the detection of IgG and IgM antibodies, specifically through Chemiluminescent Immunoassay (CLIA). Another advanced technique used to detect CMV and HHV-6 is Real-time PCR. It is the most sensitive and widely used technique for detecting CMV and HHV-6. It targets the conserved regions or genes to detect CMV and HHV-6 DNA extracted from blood, plasma, leukocytes, biopsy samples, and body fluids including urine, saliva, and cerebrospinal fluid (CSF) [4, 12]. Despite these advancements, Pakistan lacks standardized pre-donation screening protocols for CMV and HHV-6. Implementing such screening is imperative to enhance blood transfusion safety and mitigate the risk of transmitting these blood-borne pathogens to immunocompromised patients.

Building on these advanced detection techniques and the current lack of standardized screening protocols in Pakistan, this study assesses the prevalence of CMV and HHV-6 among healthy blood donors at the Regional Blood Centre in Abbottabad, Pakistan. The primary aim is to generate critical data that would enhance blood transfusion safety by evaluating demographic influences—including age and gender differences—on infection rates. Ultimately, these findings are intended to inform the development of standardized screening strategies in order to mitigate transfusion-transmitted infections, particularly among immunocompromised patients and other vulnerable recipients.

2. METHODOLOGY

2.1. Sample Collection and Ethical Compliance

This cross-sectional study was conducted from December 2021 to June 2022 at the Regional Blood Centre in Abbottabad, Pakistan. Healthy blood donors were selected as per the WHO healthy blood selection criteria [13]. A total of 1850 healthy individuals aged ≤17 years with a weight of >50 kg were recruited for blood donation. All potential donors filled a comprehensive questionnaire aimed at excluding individuals exhibiting symptoms of hepatitis, pregnant individuals, and those reporting high-risk sexual behavior within the two weeks preceding the planned donation [4, 14]. The regional Blood Center Ethics Committee Abbottabad and The University of Haripur approved the study protocol.

2.2. Detection of Antibodies against IgG and IgM of CMV and HHV-6

Plasma antibodies against CMV and HHV-6 were detected using (ELISA-VIDITEST VIDIA for HHV-6 and anti-

CMV IgG and IgM Prague, Czech Republic). The strips were immobilized with native HHV-6 antigen, which can interact with HHV-6 IgG in the tested serum, forming an antigen–antibody complex. Subsequently, introducing an animal-derived anti-human IgG antibody, coupled with horseradish peroxidase, initiated a chromogenic reaction with the substrate (TMB-O), resulting in the emission of color. This diagnostic kit holds a CE mark for *in vitro* use, boasting a manufacturer-determined sensitivity of 99% and specificity of 95%. The absorbance was measured at 450/620 nm utilizing a microplate reader (Epoch 2 Microplate spectrophotometer; BioTek, Winooski, VT, USA).

2.3. Statistical Analysis

Age groups were categorized into <18, 21-30, 31-40, and 41-50 years of age. Descriptive statistics, mean, and standard deviation (SD) were used to investigate the characteristics of the study sample. In addition, Pearson's Chi-square test was used to perform a bivariate analysis to determine the association between HHV-6 and CMV status and the age factor. The significance level was defined as $p<0.05$. Data were analyzed using Statistical Package for Social Sciences or SPSS (version 25).

3. RESULTS

This study is based on blood samples collected from a cohort of 1850 healthy male blood donors, with an average age of 28.2 years (median 27.4 years, age range 19-50 years). Approximately 100 participants were excluded due to not meeting the blood donor criteria, leaving a final sample size of 1750 healthy and asymptomatic healthy donors. Comprehensive screening of 1750 blood donors revealed a hierarchical pattern of viral and bacterial infections. Hepatitis C virus demonstrated the highest prevalence at 2.4% (42/1750), representing a significant concern for blood safety protocols. Syphilis seropositivity was detected in 1.14% (20/1750) of donors, while Hepatitis B surface antigen was present in 0.69% (12/1750). Notably, screening for malarial parasites and HIV yielded no positive cases, suggesting effective pre-donation screening measures and lower endemic rates in the study population (Table 1).

Table 1. Screening for Hepatitis B, C, Syphilis, Malaria, and HIV

Test	Donor Positive	Percentages (%)
Hepatitis B Virus	12	0.69
Hepatitis C Virus	42	2.4
Syphilis	20	1.14
Malarial Parasite	00	00
Human Immunodeficiency Virus	00	00

The analysis of blood group frequencies demonstrated an equal distribution of blood groups O and B (36.0% each, 630/1750), collectively comprising 72% of the donor population. Blood group A represented approximately one-quarter of donors (24.0%, 420/1750), while AB was the least common (4.0%, 70/1750). The Rh factor distribution showed a strong predominance of Rh-positive phenotype (96.0%, 1680/1750), consistent with global demographic patterns (Table 2).

Table 2. Distribution of A, B, O, and Rh Blood Groups

Blood Group	Number of Donors	Percentage (%)
O	630	36
A	420	24
B	630	36

Blood Group	Number of Donors	Percentage (%)
AB	70	4
Rh-positive	1680	96
Rh-negative	70	4
Total	1750	100

Serological assessment revealed a high endemicity of cytomegalovirus, with CMV-IgG antibodies present in 90.2% (1577/1750) of donors, indicating widespread past exposure. Active or recent infections, marked by CMV-IgM and HHV-6 IgM, were detected in 5.7% (100/1750) and 8.0% (140/1750) of donors respectively, suggesting ongoing viral circulation in the community (Table 3).

Table 3. Serological Assessment of CMV and HHV-6 Antibodies

Antibody Type	Number of Positive Cases	Prevalence (%)
CMV-IgG	1577	90.2
CMV-IgM	100	5.7
HHV-6 IgM	140	8

Age-stratified analysis of serological markers revealed notable variations in antibody prevalence. In donors aged <18 years (*n*=100), 80% were CMV IgG positive, while neither CMV IgM nor HHV-6 IgM was detected, suggesting a predominance of past exposure without recent infection. Among donors aged 21–30 years (*n*=1100), CMV IgG positivity was observed in 89%, with 5.45% testing positive for CMV IgM and 9.1% for HHV-6 IgM, indicating a modest level of recent viral activity. In the 31–40 year age group (*n*=520), the prevalence of CMV IgG was higher at 94.2%, accompanied by 7.69% positivity for CMV IgM and 7.6% for HHV-6 IgM. Finally, in the 41–50 year cohort (*n*=30), all donors (100%) were CMV IgG positive, with no detectable CMV IgM or HHV-6 IgM, reflecting a pattern of remote infection without current reactivation (Table 4).

Table 4. Distribution of CMV-IgM, IgG, and HHV-6 IgM among Different Age Groups

Age Group	Number of Donors	CMV-IgM Positive %	CMV-IgG Positive %	HHV-6 IgM Positive %
< 18	100	0 (0/100)	80 (80/100)	0 (0/100)
21-30	1100	5.45(60/1100)	89 (980/1100)	9.1 (100/1100)
31-40	520	7.69 (40/520)	94.2 (490/520)	7.6 (40/520)
41-50	30	0 (0/30)	100 (30/30)	0 (0/30)

4. DISCUSSION

This study assessed the prevalence of CMV and HHV-6 infection among the adult population of Abbottabad, Pakistan. Similar results were presented by another study which evaluated the prevalence of CMV infection in the nation's adult population. There was found a significant seroprevalence of CMV infection, with 93.2% of IgG antibodies, 4.3% of IgM antibodies, and 95% of IgM-positive individuals also having IgG antibodies [15]. This is higher than those reported from North America, Europe, Australia, and Africa [16-19], but consistent with the research results from Asia, South America, and the Caribbean . A study conducted in Nigeria reported CMV infection in 96.2% of the population. Anti-CMV IgG antibodies were found to be common in 96.2% of the population, but IgM antibodies were common in just 2.6% [20, 21].

CMV seroprevalence is typically higher in women, older age groups, people from low socioeconomic backgrounds, and developing nations [15–22]. Similarly, in a study conducted in Lahore, Pakistan, 97.8% of blood donors exhibited detectable CMV-specific IgG antibodies, indicating a high seroprevalence rate [24]. In this investigation, elevated levels of CMV IgM antibodies were observed in individuals aged 31-40. Conversely, the prevalence of CMV IgG antibodies demonstrated a peak of 100% in the 41-50 age group, followed closely by the 31-40 age group at 94% [23, 25]. The, current results are consistent with those of other researches from regions with high seroprevalence, where more than 90% of people in the 18-35 age range were found to have a positive CMV-IgG response [26].

An a study from blood donor population in Madinah, Saudi Arabia in 2022 revealed that they were seropositive for CMV. Donors in the age groups of 18-30, 31-40, and 41-60 were selected from the Central Blood Bank in Madinah. Anti-CMV IgG and IgM antibodies were tested using ELISA. IgG positivity was determined to be 95% based on the results. Only 1.6% of donors tested positive for IgG and IgM antibodies [14].

In this cross-sectional study of 1750 blood donors from diverse socioeconomic backgrounds in Abbottabad, Pakistan, we assessed CMV seroprevalence through anti-CMV IgM testing. The analysis revealed that 100 (5.7%) donors were positive for CMV-specific IgM antibodies, indicating recent or active infection. This IgM seroprevalence of 5.7% was notably higher than the rates reported in several other nations, though comparable to the previously published regional data. The findings suggest significant CMV transmission patterns in this geographic area, potentially influenced by varying socioeconomic and hygiene conditions [27]. The prevalence of IgM and IgG anti-CMV antibodies indicates that transfused blood products may contain a potentially contagious virus [28].

According to an in-depth research and meta-analysis, the prevalence of anti-CMV IgG and IgM antibodies among blood donors worldwide is 83.16% and 13.77%, respectively. Approximately 23.78% of blood donors globally exhibit anti-CMV IgM and IgG antibodies [29]. Immunocompromised patients who received contaminated blood products are significantly more likely to develop CMV infection and die from it [30, 31]. Leukoreduction and choosing blood products from donors who tested negative for CMV are alternate methods to stop the spread of TT-CMV through blood transfusion [7, 32]. Among 175 samples analyzed to check the seroprevalence of HHV-6 IgM antibodies in healthy blood donors of different ethnicities, 14 (8%) tested positive for IgM.

The analysis of HHV-6 seroprevalence across age groups revealed that 8% of the total donor cohort had detectable HHV-6 IgM antibodies. Age distribution showed significant variation ($p < .001$), with peak seropositivity of 9.1% observed in donors aged 21-30 years, followed by 7.6% in the 31-40 age group. Notably, no HHV-6 IgM antibodies were detected in donors younger than 20 years or those aged 41-50 years. These age-specific patterns align with global data showing geographic variation in transfusion-associated HHV-6 transmission risk [4, 33] . Despite the high seropositivity reported in previous studies, HHV-6 DNA is rarely detected in seropositive individuals. While transmission risk exists, broad HHV-6 testing is not currently warranted. Further

research using a standardized detection method is needed to assess transmission risk from seropositive donors with high viral loads [11]. In a 2015 study in Greece, 25% of randomly selected blood donors tested positive for HHVs using PCR. One sample showed exceptionally high HHV-6 DNA concentration. None of the Greek donors had HHV-8, although 78.1% had HHV-6. Gender differences were not found to be significant. The study concluded that HHV-8 transmission risk was low, while HHV-6 posed a substantial risk to transfusion safety in Greece [11].

4.1. Limitations

Our findings should be interpreted considering several limitations. Firstly, our sampling of healthy blood donors may introduce selection bias, potentially underestimating true population prevalence rates. Secondly, the single-center design in Abbottabad limits generalizability to other Pakistani regions, particularly rural areas with varying socioeconomic and healthcare profiles. Thirdly, the cross-sectional nature of our study precludes assessment of temporal changes in viral seroprevalence patterns. Additionally, it also prevents the establishment of causal relationships, while the absence of viral load data means the transmission risk via blood transfusion remains uncertain. Moreover, although IgM and IgG antibodies are useful indicators, they do not definitively confirm active infection or viral shedding. On the contrary, the study's strength lies in its large sample size, which strengthens the reliability of the results. Further, the findings are consistent with international studies, boosting confidence in their validity. By analyzing both IgM and IgG antibodies, the current study provides a more comprehensive understanding of infection dynamics, which is crucial to assess transfusion safety. Hence, despite limitations, the research provides valuable data on CMV and HHV-6 prevalence and transfusion-related risks, though further studies—particularly focusing on viral loads—are needed to fully evaluate the implications for blood transfusion practices.

4.2. Conclusion and Implications

This study sheds light on the substantial prevalence of CMV and HHV-6 among healthy blood donors in Abbottabad, emphasizing the critical importance of screening for these transfusion-transmitted pathogens. The findings revealed a noteworthy seroprevalence of CMV IgG antibodies, indicative of prior infection, while both CMV and HHV-6 IgM antibodies demonstrated elevated seroprevalence rates among volunteer blood donors. These results underscore the necessity of incorporating HHV-6 and CMV testing as routine components of pre-transfusion screening protocols to minimize the risk of associated complications. Moreover, the study advocates adopting molecular techniques to assess blood donors for these viral infections.

CONFLICT OF INTEREST

The authors of the manuscript have no financial or non-financial conflict of interest in the subject matter or materials discussed in this manuscript.

DATA AVAILABILITY STATEMENT

The data associated with this study will be provided by the corresponding author upon request.

FUNDING DETAILS

No funding has been received for this research.

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